云 南 植 物 研 究 1994; **16** (3): 255—262
Acta Botanica Yunnanica

探讨云南山茶起源的一线曙光——野生二倍体类型在金沙江流域的发现*

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摘要 新近发现的云南山茶(*Camellia reticulata*)原始二倍体类型分布于金沙江河谷流域的云南华坪县及四川盐边县，海拔 1800—2800 m。其植被类型为亚热带常绿阔叶林与云南松的混交林，生境较为湿润。云南山茶原始二倍体类型的形态特征与广布的六倍体类群非常相似，而与怒江山茶及西南山茶的形态特征不同。二倍体的花粉母细胞在减数分裂中期 I 形成 15 个二价体，间期的细胞核结构为球形前染色体型，分裂前期染色体为中间型，其核型公式为 2n＝30＝22m+2m(SAT)+4sm+2st。根据本文和前人的研究结果，作者对云南山茶的形成和演化进行了讨论，否定了云南腾冲是云南山茶的原产地的观点，并认为二倍体类型的云南山茶与怒江山茶 (*C. saluenensis*)，西南山茶(*C. pitardii* var. *pitardii*)经过多次杂交和连续多倍化形成一个多倍体复合体，云南山茶的二倍体类型与怒江山茶及西南山茶可能是六倍体云南山茶的共有祖先。

关键词 云南山茶，二倍体类型，形态特征，细胞学

DAWN ON THE ORIGIN OF CAMELLIA RETICULATA ——THE NEW DISCOVERY OF ITS WILD DIPLOID IN JINSHAJIANG VALLEY

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Abstract The newly discovered diploid type of *C. reticulata* was distributed in Jinshajiang valley with an elevation of 1650— 2800 m, which is situated in Huaping of Yunnan province and Yanbian of Sichuan province. This diploid occured in the habitat with higher humidity. Its vegetation was mainly consisted of evergreen broad-leaved forest and *Pinus yunnanensis* forest. The morphologic characters of this diploid type were very similar to those of its hexaploids. Its chromosomes in pollen mother cells formed 15 bivalents at metaphase I. The resting nuclei in somatic cells were of the round prochromosome type, the somatic chromosomes at mitotic

* 云南省自然科学基金资助项目，** 通讯联系人
1993-07-22 收稿，1994-04-18 修回

prophase were of the interstitial type. The karyotype at somatic metaphase was formulated as: 2n = 30 = 22m+2m(SAT)+4sm+2st. The discovery of diploid types of *C. reticulata* negates the hypothesis that Tengchong county of Yunnan province is the original place of *C. reticulata*. Based on the results reported in this paper and by the previous authors, it was considered as follows: 1) *C. reticulata* forms a polyploid complex with its closely related diploid species *C. saluenensis* and *C. pitardii* var. *pitardii;* 2) the hexaploid *C. reticulata* was possibly resulted from interspecific hybridization and continued polyploidization among its ancient diploid species; 3) the newly discovered dilpoid of *C. reticulata* and the above two diploid species were probably the foundation of this polyploid complex.

Key words *Camellia reticulata,* Diploid type, Morphological characters, Cytology

INTRODUCTION

The Yunnan camellia, *Camellia reticulata* Lindley, has been cultivated in China for more than 1300 years, and is famous in the world for its large flower size, bright and charming colors, many cultivars and a long flowering season. This species occur in some parts of southwestern China with an elevation of 1800—2500 m. The scientific name, *Camellia reticulata* Lindley, was originally bestowed in 1827 on plants with semidouble flowers which were introduced to West from China respectively by Captain Rawes in 1820 and by John Damper Parks in 1824 [1] . During the period from 1904 to 1934, George Forrest collected a number of plants with single flowers of *C. reticulata* from Tengchong county, Yunnan, China, which were treated as a form of *C. reticulata,* namely, *C. reticulata* f. *simplex* by Sealy [2] . Sealy considered the plants with single flowers as the original form of *C. reticulata.* Later, some authors supported Sealy's opinion [3] and considered that Tengchong was the original distribution place of *C. reticulata,* while some other authors speculated that *C. reticulata* was only a garden form derived from *C. pitardii* var. *yunnanica* [4] .

In 1970s, Ackerman [5] and Kondo [6] found that *C. reticulata* was a hexaploid plant with 2n = 90 chromosomes (the basic chromosome number of the genus is x = 15). Parks and Griffiths [7] studied the *C. saluenensis–pitardii–reticulata* complex biosystematically and found them to be interrelated. After that, McClung [8] stated that the form of *C. reticulata, C. saluenensis* and *C. pitardii* found in Western and Chinese gardens may be complex hybrids involving two or more of the species as well as fractionally combined with *C. japonica.*

In 1980s, in order to clarify the origin and evolution of *C. reticulata,* an extensive cytological studies were carried out in the wild and cultivated *C. reticulata,* especially the semidouble and double as well single forms collected from Tengchong county by authors in this paper. It was found that all the above *C. reticulata* were hexaploids with 2n = 90 chromosomes [5,6,9,10] and that most *C. reticulata* populations from Yunhua of Tengchong county formed 45 bivalents at metaphase I, besides one white–flowered cultivar forming various valents [11] .

Recently, an extensive investigation on *C. reticulata* was made throughout its whole natural range, especially in the Jinshajiang valley where a lots of plants of section *Camellia* were found. According to our observation, *Camellia reticulata* distributed in Tengchong possesses semidouble and double as well as single forms. In Huaping county of Yunnan province and Yanbian county of Sichuan province, the plants were morphologically similar to single form of *C. reticulata* in Tengchong, and over there some diploid

types of *C. reticulata* were detected. In this paper, the distribution area, habitat, morphological and cytological characters of *C. reticulata*, especially its newly discovered diploid types were reported, in addition, the origin and evolution of *C. reticulata* were discussed.

RESULTS AND DISCUSSIONS

1.Distribution and habitat

According to our recent investigation, *C. reticulata* are distributed in most parts of Yunnan province, the western part of Guizhou province and the southern part of Sichuan province (Fig.1).

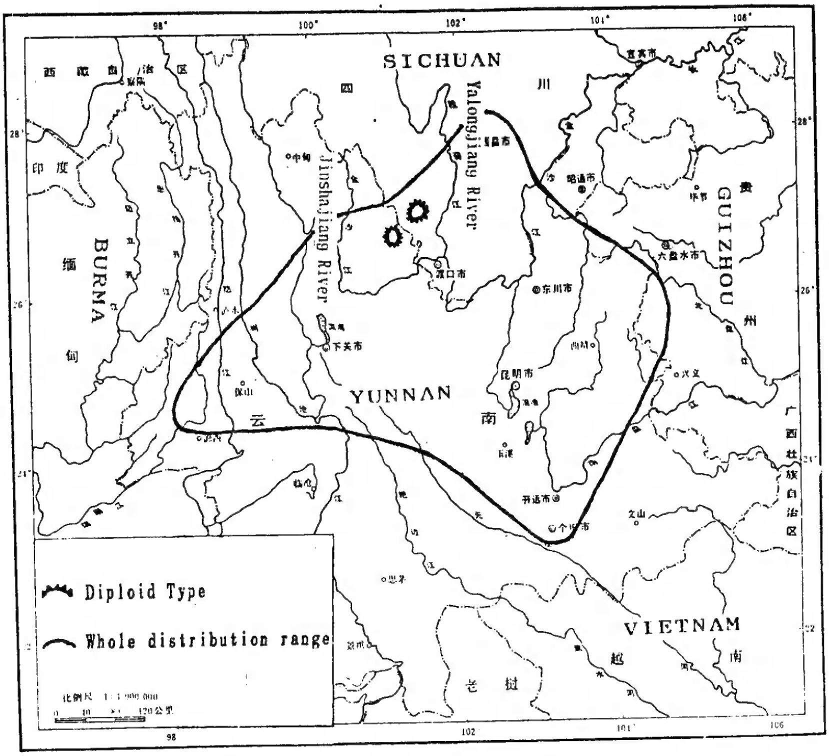


Fig. 1 The distribution of *C. reticulata*

As Figure 1 exhibited, the diploid types of *C. reticulata* were distributed in a relatively restricted area of Huaping county, Yunnan province and Yanbian county, Sichuan province. In contrast to its diploid types, the tetraploid types were distributed in a broader area including Xichang, Dechang, Miyi, Huili, Huidong, Ningnan, Puge and Yanbian county etc. of Sichuan province (to be published in other paper), while the hexaploid types were distributed in most parts of southwestern China.

Like other *Camellia* plants, this diploid grows within the range of elevation of 1650—2500 m, and usually occur in the valley with high humidity and in the northern and northwestern slope of mountain, and often co-occur with *Rhododendron decorum*, *R. delavayi* and *Ternstroemia* sp. etc. The upper layer trees are commonly consisted of evergreen broad-leaved trees e.g. *Castanopsis delavayi*, *C. orthacantha*, *Cyclobalanopsis glaucoides*, *C. delavayi*, *Quercus franchetii*, *Cinnamomum glanduliferum* and coniferous

trees e.g *Pinus yunnanensis*.

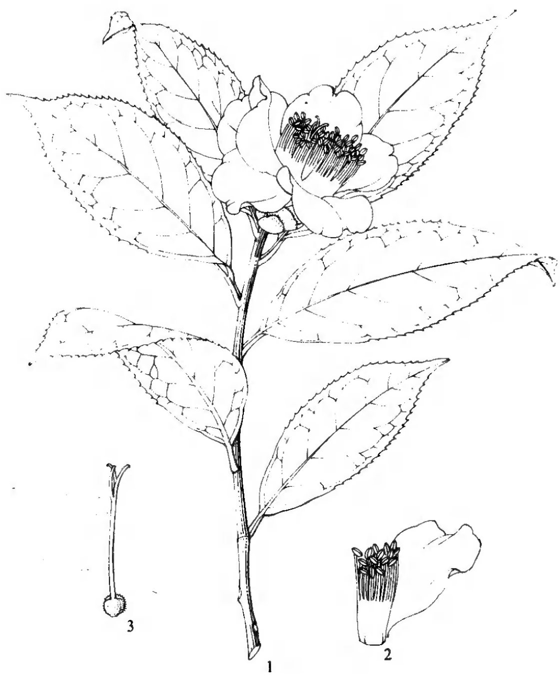


Fig 2 The diploid type of *Camellia reticulata* Lindl.
1. flowering shoot; 2. petal and androecium;
3. gyncium(drawed by Xiao Rong)

2.Morphological characters

As figure 2 showed, morphologically the diploid types of *C. reticulata* were similar to its tetraploids(to be published) and hexaploids. The characters of diploid types were descripted as follows: a loosely branched small tree, leaves blades broad elliptic or elliptic, few oblong-elliptic, rarely suborbicular, acute to acuminate, rarely caudate with the tip up to 1 cm long, base cuneate, rarely wide cuneate to rounded, (6.5)—7—12 cm long, (2.3)—3.0—5.5 cm wide, regularly serrulate, midrib raised below, venation clearly visible on both surface; petioles stout, 0.8—1.2 cm long, coarsely black hairs. Flowers solitary or geminate, sometimes 3, at the end of the branches, also in the axils of the leaves. Corolla 4—6 cm long, about 4.5 cm across, red, consisting of 5—6 petals adnate to the androecium for up to 1.1 cm from the base. Androecium 2.5—3.4 cm long, glabrous to villose; outer filaments united 1/2 to 3/4 their length from the base, pale yellowish. Gynoecium 2.4—3.6 cm long; ovary globose, about 4 mm long, white tomentose; style 2—3 cm long, divided into 3—5 arms at the apex. Capsule oblate, 4.5—6.2 cm high and 4.8—6.5 cm in diameter, red(especially at the base) to light brown, rough, scarcely villose on the surface, 3—5 locular with 1—3 seeds in each loculus, splitting into 3—5 valves; wall up to 1.8 cm thick when fresh, and 0.7—1.4 cm thick when dry, woody. Seeds 1.2—1.4 cm long, 1.3—1.7 cm wide, dull brown.

Morphologically it is easy to distinguish the diploid type of *C. reticulata* from its related diploid spcecies. The main differences between them were listed in table 1.

As showed in table 2, all three kinds of ploids of *C. reticulata* collected respectively from four places displayed foveolate-reticulate sculpture of pollen exine, despite their different sizes of lumina on the surface of exine. Both *C. pitardii* and *C. saluenensis* showed rugulate-fossulate sculpture, which was different from that of *C. reticulata*. (plate II:4,6).

3. Cytological characteristics of newly discovered diploids

The materials used here were collected from Huaping county of Yunnan province and Yanbian county of Sichuan province. This area has an elevation of 1800—2800 m. The voucher specimens (L.F. Xia et al. 902030, 902031, 902032, 902034, 902035, Z.L.Wang et al. 920204) were deposited in the Herbarium of Kunming Botanical Garden, the Academy of Sciences of China. Flower buds were directly fixed in Carnol's solution II(ethanol:glacial acetic acid:chloroform = 6:3:1) for meiosis observation. Root-tips

from germinated seeds were used for karyotype observation. Six populations were investigated, two of which the individual comparisons were carried out. Totally more than 100 cells were observed. Karyotype formulas were based on the average data of measurements of somatic chromosomes at metaphase from five cells. Very similar karyotype were detected among populations or individuals. The resting nuclei in somatic cells were of the round prochromosome type (plate I: A), and the somatic chromosomes at mitotic prophase were of the interstitial type (plate I: B), these results were the same as those reported in other camellias by previous authors [9] . The karyotype formulas of the diploid forms were 2n = 30 = 22m+2m(sat)+4sm+2st, the 17th, the 18th, the 23rd and 24th chromosomes were submedian centromeric chromosomes, while the 25th and 26th chromosomes were subterminal centromeric chromosomes, two out of 24 median-centromeric chromosomes, i.e. the 6th and the 21st chromosomes showed satellites on their short arms (plate I: E), the karyotype asymmetry was categorized to be "2A type". At diakinesis and metaphase I chromosomes formed 15 bivalents in the pollen mother cells (plate I: C). The chromosome paring was regular, no any other valents was detected.

Table 1 The main character comparisons between the diploid type of *C. reticulata* and its related diploid species

species	Leaves		Flowers	Capsules	
	Shape	Length × Width (cm)	Diameter Ovary (cm)	Diameter (cm)	Wall
C. reticulata	broad elliptic or elliptic	(7—12) × (3.0—5.5)	4.5—5.5 tomentose	4.8—6.5	1.3—1.7 cm thick, rough
C. saluenensis	elliptic, acute to obtuse,	(3—5.5) × (1—2.3)	2.5—4.0 tomentose	2.3—3.0	0.1—0.3 cm thick, rough
C. pitardii	oblong-elliptic, abruptly acuminate or caudate, prominently serrulate	(6.5— 10) × (2.2—3.5)	3.5—4.5 tomentose	3.5—5.0	0.1—0.3 cm thick, rough
C. japonica	elliptic	(6.5— 8.0) × (3.0—4.0)	3.3—4.5 glabrous	2.2—3.2	0.2—0.3 cm thick, smooth

4.Discussion

(1) Besides hexaploid, there are tetraploid and diploid types in *C. reticulata*. The morphological characters of the newly discovered diploid types studied here were very similar to those of the tetraploids and hexaploids of *C. reticulata*, furthermore all the three ploidies of *C. reticulata* displayed similar sculpture of pollen exine. The only differences among them was that the diploid types possessed thickest capsule walls and more or less villose on its filaments. These facts revealed that there were a series of polyploids in *C. reticulata*, and the variation was mainly caused by ploidy difference.

(2) As table 1 showed, morphologically it was easy to distinguish the diploid type of *C. reticulata* with its closely related diploid species *C. pitardii*, *C. saluenensis* and *C. japonica* by comparing their characters of leaves, flowers and capsules (see table 1) as well as their sculpture of pollen exine(see table 2). This information meant that there were a series of forms with different and nonintergrading morphological characters on the same basic ploidy level [12] . Geographically *C. saluenensis* is widely spread in Yunnan province and southern Sichuan province, while *C. pitardii* is widely distributed in Guizhou province and

northeastern and southern Sichuan province. In comparison with its allied diploid species, the new discovered diploid type of *C. reticulata* have a relatively restricted distribution, occurring only in the mountains with elevation of 1650—2600 m, adjacent to the distribution of its newly discovered tetraploids, moreover its geographical range were partly overlapped with that of the aforementioned two diploid species. Whereas the hexaploid *C. reticulata* have widespread and continuous distributions (see Figure 1). Cytologically, the diploids of *C. reticulata* and its closely related diploid species had 30 chromosomes in the somatic cells and formed 15 bivalents at diakinesis and metaphase I despite their karyotypical difference, while most of newly discovered tetraploid *C. reticulata* had 60 chromosomes in somatic cells and formed 30 bivalents at metaphase I (to be published). Panzhihua of Sichuan province is connected with the distribution area of the newly discovered diploids and tetraploids, *C. reticulata* collected from Panzhihua had 90 chromosomes in the somatic cells and formed 45 bivalents(to be published), which was the same as those from Tengchong of Yunnan province [11]. Quite often a polyploid complex consists of a series of distinct diploid species which have hybridized and become polyploid to produce a range of tetraploid, hexaploid and sometimes higher levels of ploidy [13]. So it could be concluded that: 1) the tetraploids and hexaploids of *C. reticulata* were genomic allopolyploids; 2) the diploids, tetraploids as well as hexaploids of *C. reticulata* formed a polyploid complex with its related diploid species; 3) all the above diploids were the probable foundations of this polyploid complex. 4) Tengchong county of Yunnan province was not the original place of *C. reticulata*.

Table 2 Pollen morphological data

Taxa and Chr. No.	Voucher	Location	Pollen shape, exine sculpture and pollen size(um)	Plate No.
C. reticulata 2n=90	Xia,L F et al. 44	Tengchong Yunnan	prolate, foveolate-reticulate (55.4—66.1)61.9×34.7(32.6—36.4)	II:1,2
C. saluenensis 2n=30	Wang,Z L 910203	Kunming Yunnan	prolate,rugulate-fossulate (30.9—47.2)40.2×23.6(20.2—28.4)	II:3,4
C. pitardii 2n=30	Wang,Z L 890202	Yiliang, Yunnan	prolate,rugulate-fossulate (40.2—52.3)44.4×26.5(23.6—29.8)	II:5,6
C. reticulata 2n=60	Gu,Z J et al. 91127	Xichang, Sichuan	subprolate,foveolate-reticulate (39.8—50.8)41.3×32.6(31.2—34.7)	II:7,8
C. reticulata 2n=90	Wang,Z L 009	Panzhihua, Sichuan	prolate,foveolate-reticulate (50.8—59.3)53.4×37.7(34.7—39.8)	II:9,10
C. reticulata 2n=30	Xia,L F et al. 902034	Yanbian, Sichuan	subprolate,foveolate-reticulate (41.2—48.3)44.9×34.2(33.1—35.8)	II:11,12

(3) Interspecific hybridization is now accepted as a major mechanism for generating evolutionary novelty in the plant kingdom [14]. Recent evidence has confirmed this mechanism in genus *Senecio* and genus *Helianthus* by isozymic examination and restriction site analysis of chloroplast and ribosomal DNA [15]. In relation to the question of how natural polyploids arise, it is now clear that the most common mechanism of polyploid evolution is that 2n gamete production, i.e. sexual polyploidization as a result of the union of 2n gametes to produce a tetraploid and subsequent cross between the tetraploid and 2n gametes produced by diploids [16]. According to his extensive studies of crossing compatibility in genus *Camellia*, Parks [4] pointed out that the hexaploids of *C. reticulata* could be easily crossed with *C. pitardii* and *C. saluenensis*, furhtermore he also speculated that *C. reticulata* possibly formed a complex with *C.*

saluenensis, *C. pitardii* and *C. japonica*. In addition, the researches of DNA fingerprint by using RAPD DNA marker revealed that *C. reticulata* showed some typical bands of *C. saluenensis* and *C. pitardii* var. *pitardii*, which also indicated that *C. reticulata* contained some genetic components of *C. saluenensis* and *C. pitardii* var. *pitardii* (Xiao Tiaojiang et al, to be published). Thus, based on all the results presented here and reported by the previous authors, the hexaploid *C. reticulata* was possibly resulted from interspecific hybridization and continued polyploidization among its ancient diploid species. The evolutionary relationships among diploids and polyploids in this complex could be illustrated in Figure 3.

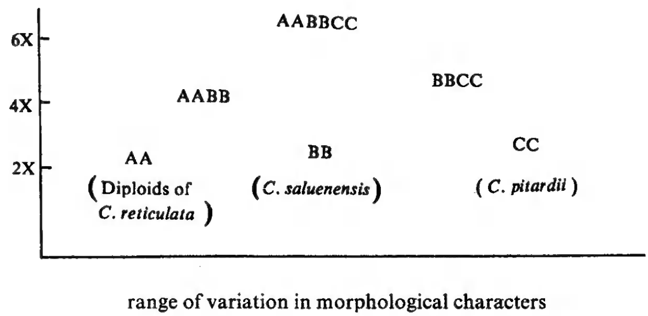


Fig. 3 The possible structure of the polyploid complex
AA = the diploid type of *C. reticulata*; BB = *C. saluenensis*,
CC = *C. pitardii* var. *pitardii*; AABBCC = the hexaploid type of *C. reticulata*

In order to verify the above structure, furthermore researches are necessary of electrophoretic isozyme markers and variation in nuclear and chloroplast genomes and the biosystematic analysis.

Acknowledgement The authors would like to express their thanks to Prof. TANG Yan-Cheng for his advice and valuable comments on the first manuscript.

Literature cited

[1] Yu D J. A historical review and future development of *Camellia reticulata* in Yunnan. *Acta Hort Sinica*, 1985, **12**(2):131—136(in Chinese).

[2] Sealy J R. A revision of the genus Camellia. London: Roy Hort Soc, 1958.

[3] Feng G M, Xia L F, Zhu X H. Yunnan Camellias of China. Bejing: Science Press, 1986.

[4] Parks C R. Cross-compatibility studies in the genus Camellia. *International Camellia Journal*, 1990, **10:** 37—54 .

[5] Ackerman W L. Genetic and Cytological Studies with Camellia and Related genera. Technical Bull, 1971, 1427 USDA.

[6] Kondo K. Chromosome Numbers in the Camellia. *Biotropica*, 1977, **9**(23):86—94 .

[7] Parks C R, Griffiths A. The saluenensis-pitardii-reticulata complex. *Camellia Review*, 1963, **25**(2):12—29.

[8] McClung J H. The Camellia family--Section A. Camellia species. In: Feathers D L, Brown M H. eds, The Camellia. *Am Camellia Soc*, 1978, 476.

[9] Gu Z J, Xia L F et al. Report on the chromosome numbers of some species of Camellia in China. *Acta Botanica*

Yunnanica, 1988, **10**(3):291—296.

〔10〕 Kondo K, Gu Z J et al. A Cytological study of *Camellia reticulata* and its related species in Yunnan,China. *La Kromosome* II, 1986, **43—44:** 1405—1419.

〔11〕 Xiao T J, Gu Z J, Xia L F. A study of meiosis of 9 species in genus Camellia. *Acta Botanica Yunnanica,* 1993, **15**(2):167—172.(in Chinese) .

〔12〕 Grant V. Plant speciation (2nd edn). New York: Columbia University Press. 1981.

〔13〕 Stace C A. Plant taxonomy and biosystematics. University Park, Baltimore. Maryland, USA, 1980.

〔14〕 Abbott R J, Plant invasions, interspecific hybridization and the evolution of new plant taxa. *TREE,* 1990, **7**(12):401—405

〔15〕 Abbott R J, Forbes D G. Outcrossing rate and self-incompatibility in the colonizing species Senecio squalidus. *Heredity,* 1993, **71:**155—159.

〔16〕 Thompson J D, Lumaret R. The evolutionary dynamics of polyploid plants:origin, establishment and persistence. *TREE,* 1992, 7(9): 302—307.

Explanation of Plates

Plate I Representative of chromosome of diploid type of *C. reticulata*

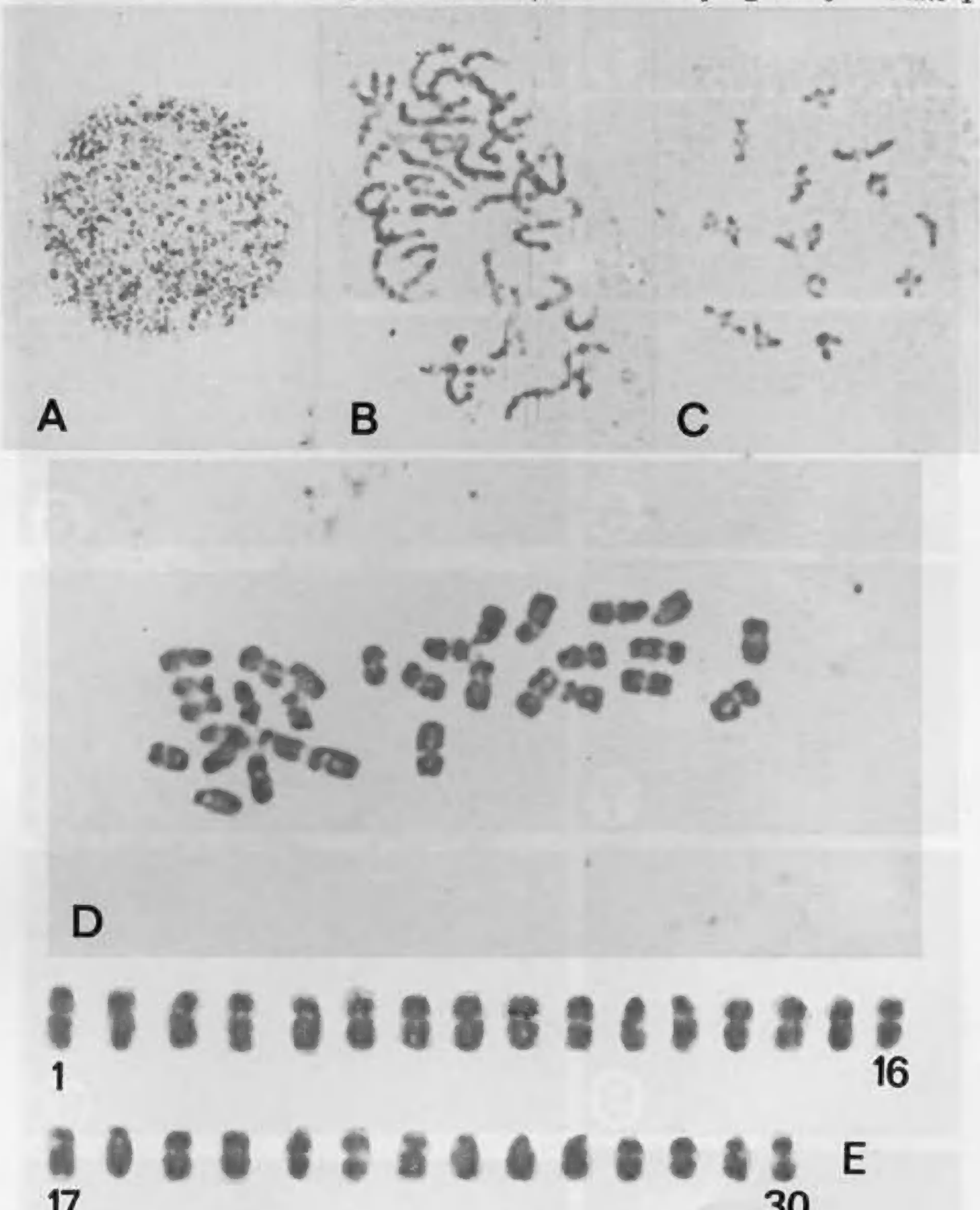
A. resting stage; B. Mitotic prophase; D. Mitotic metaphase; E. Aligement of the chromosome at metaphase in somatic cell;C. meiotic chromosome form 15II at metaphase I, in PMC

Plate II SEM photographs of the pollen grains of *Camellia*

1-2. *C. reticulata* from Tengchong; 7-8. *C. reticulata* from Xichang 3-4. *C. saluenensis* from Kunming; 9-10. *C. reticulata* from Panzhihua 5-6. *C. pitardii* from Yiliang; 11-12. *C. reticulata* from Yanbian

夏丽芳等：探讨云南山茶起源的一线曙光——野生二倍体类型在金沙江流域的发现　　图版 I

XIA Li-Fang et al: Dawn on the Origin of *Camellia reticulata* ——The New Discovery of Its Wild Diploid in Jinshajiang Valley　　Plate I



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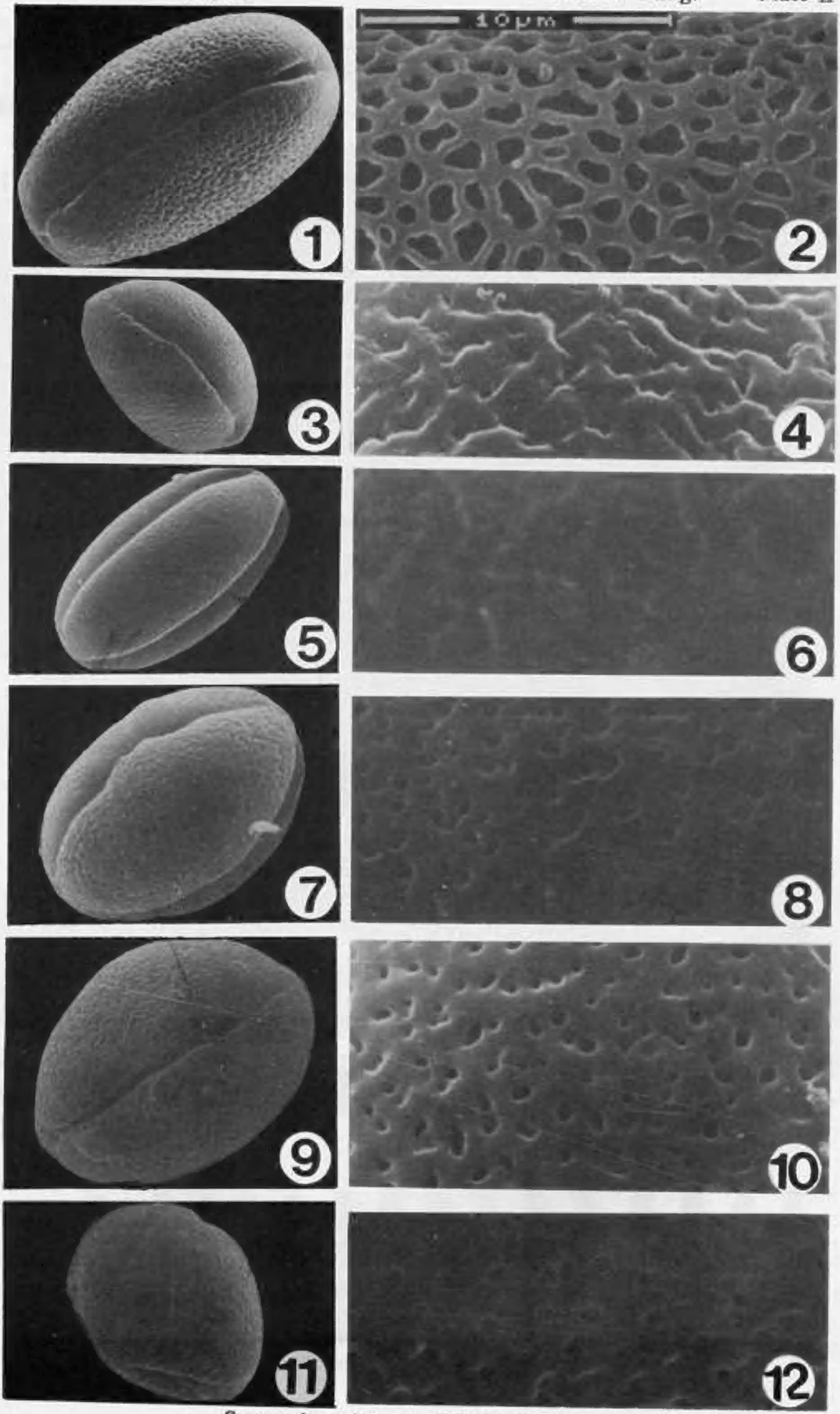

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